CLAIMS

1. A detection method for detecting a variation in *GH1* effective to act as an indicator of GH dysfunction in an individual, which detection method comprises the steps of:

(a) obtaining a test sample comprising a nucleotide sequence of the human *GH1* gene from the individual; and

(b) comparing the sequence obtained from the test sample with a standard sequence known to be that of the human *GH1* gene, wherein a difference between the test sample sequence and the standard sequence indicates the presence of a variation (hereinafter "variant of *GH1*") effective to act as an indicator of GH dysfunction

wherein the test sample is obtained from an individual exhibiting the following criterion:

(i) growth failure, defined as a growth pattern [delineated by a series of height measurements; Brook CDG (Ed) Clinical Paediatric Endocrinology 3rd Ed, Chapter 9, p141 (1995, Blackwell Science)] which, when plotted on a standard height chart [Tanner *et al* Arch Dis Child 45 755-762 (1970)], predicts an adult height for the individual which is outside the individual's estimated target adult height range, the estimate being based upon the heights of the individual's parents.

2. A method according to claim 1, wherein the test sample is obtained from an individual exhibiting at least one of the following further criteria:

(ii) height velocity below the 25th centile for age; and/or

(iii) bone age delay according to the Tanner-Whitehouse scale of at least two years when compared with chronological age; and/or

(iv) no other disorder known to cause inclusion in criteria (i) to (iii) above.

3. A method according to claim 2, wherein the bone age delay is in the range of from 2 to 4 years, when compared with chronological age.

4. A method according to any preceding claim, wherein the individual exhibits normal results in a standard growth hormone function test.

5. A method according to any preceding claim, wherein the detection method comprises any sequencing method for determining the sequence of the *GH1* gene of an individual.

6. A method according to any preceding claim, wherein the detection method comprises PCR amplification of the *GH1* gene of the individual using (a) a *GH1* gene-specific fragment, being a fragment unique to the *GH1* gene whose sequence is not found in the four other paralogous (non-*GH1*) genes in the GH cluster, and (b) one or more *GH1* gene-specific primers which cannot bind to the homologous flanking regions in the four other paralogous (non-*GH1*) genes in the GH cluster.

7. A method according to any preceding claim, wherein the detection method comprises PCR amplification of the entire *GH1* gene of the individual and nested PCR of overlapping constituent fragments of the *GH1* gene of the individual.

8. A method according to any preceding claim, wherein the detection method comprises PCR amplification of all or a fragment of genomic DNA spanning the Locus Control Region of the *GH1* gene.

9. A method according to any preceding claim, wherein the detection method comprises mutational screening of all or a fragment of the individual's *GH1* gene by DHPLC.

10. A detection method for detecting a variation in *GH1* effective to act as an indicator of GH dysfunction in a individual, which detection method comprises the steps of:

(a) obtaining a test sample comprising a nucleotide sequence of the human *GH1* gene from the individual; and

(b) comparing the sequence obtained from the test sample with a standard sequence known to be that of the human *GH1* gene, wherein a difference between the test sample sequence and the standard sequence indicates the presence of a variation (hereinafter "variant of *GH1*") effective to act as an indicator of GH dysfunction which detection method further comprises

(c) PCR amplification of the *GH1* gene of the individual using (a) a *GH1* gene-specific fragment, being a fragment unique to the *GH1* gene whose sequence is not found in the four other paralogous (non-*GH1*) genes in the GH cluster, and (b) one or more *GH1* gene-specific primers which cannot bind to the homologous flanking regions in the four other paralogous (non-*GH1*) genes in the GH cluster.

11. A detection method according to any preceding claim, which detection method further comprises the use of one or more primer(s) selected from:

CTC CGC GTT CAG GTT GGC (GH1DF);
AGG TGA GCT GTC CAC AGG (GH1DR);
GGG CAA CAG TGG GAG AGA AG (GH2DF);
CCT CCA GGG ACC AGG AGC (GH2DR);
CAT GTA AGC CCA GTA TTT GGC C (GH3DF);
CTG AGC TCC TTA GTC TCC TCC TCT (GH3DR);
GAC TTT CCC CCG CTG GGA AA (GH4DF);
GGA GAA GGC ATC CAC TCA CGG (GH4DR);
TCA GAG TCT ATT CCG ACA CCC (GH5DF);
GTG TTT CTC TAA CAC AGC TCT C (GH5DR);
TCC CCA ATC CTG GAG CCC CAC TGA (GH6DF)
CGT AGT TCT TGA GTA GTG CGT CAT CG (GH6DR);
TTC AAG CAG ACC TAC AGC AAG TTC G (GHD7F);
CTT GGT TCC CGA ATA GAC CCC G (GH7DR);
GTGCCCCAAGCCTTTCCC (LCR15: 1159-1177);
TGTCAGATGTTCAGTTCATGG (LCR13: 1391-1412);
CCTCAAGCTGACCTCAGG (LCR25: 1346-1363);
GATCTTGGCCTAGGCCTCG (LCR23: 1584-1602);
LCR 5A (5' CCAAGTACCTCAGATGCAAGG 3');
LCR 3.0 (5' CCTTAGATCTTGGCCTAGGCC 3');
LCR 5.0 (5' CCTGTCACCTGAGGATGGG 3');
LCR 3.1 (5' TGTGTTGCCTGGACCCTG 3');
LCR 3.2 (5' CAGGAGGCCTCACAAGCC 3');
LCR 3.3 (5' ATGCATCAGGGCAATCGC 3');
GH1G5 (5' GGTACCATGGCTACAGGTAAGCGCC 3');

GH1G3 (5' CTCGAGCTAGAAGCCACAGCTGCCC 3');
BGH3 (5' TAGAAGGCACAGTCGAGG 3');
GH1R5 (5' ATGGCTACAGGCTCCCGG 3'); and
GH1R3 (5' CTAGAAGCCACAGCTGCCC 3').

12. A variant of *GH1*, which differs from *GH1* and is detected by or is detectable by a method according to any preceding claim but was not detected by methods used hitherto, such as those reliant on patient selection criteria based primarily on absolute height.

13. A variant of *GH1*, which variant is selected from those characterised as unpublished in Table 7B herein "Growth Hormone deficiency; *GH1* gene mutations and polymorphisms".

14. A variant of *GH1* according to any preceding claim comprising a missense mutation.

15. A variant of *GH1* according to any preceding claim comprising a silent mutation which affects the activity of the signal peptide.

16. A variant of *GH1* comprising one or more of the following *GH1* promoter mutations:

Promoter mutation	**Associated haplotype**
A→G –248	1
T→C –495	1
A→G –177	1
T→C –30 (TATA)	1
A→G –24	1
C→T –347, A→G –44	1
A→G +62	1
G→A –48, A→G –498	2
T→C –508	2
ΔGGGGG –57 to –61	2
ΔG –57	2

17. A protein or amino acid sequence encoded by a variant of *GH1* according to any of claims 12 to 16.

18. A human GH variant, which variant is selected from the following amino acid substitutions with respect to wild type/GH:
Met→Val -26; Thr→Ala -20; Leu→Pro -12; Leu→Pro -11; Phe→Leu 1; Ile→Val 4; Asp→Asn 11; Gln→Arg 2 2; Asp→Val 26; Glu→Gly 30; Lys→Arg 41; Ser→Leu 43; Glu→Gly 56; Arg→Gly 64; Ser→Phe 71; Glu→Lys 74; Ser→Pro 85; Trp→Arg 86; Gln→Leu 91; Asp→Gly 107; Ser→Cys 108; Ser→Arg 108; Val→Ile 110; Tyr→His 143; Ala→Val 155; Leu→Pro 163; Lys→Arg 168; Lys→Glu 168; Thr→Ala 175; and Phe→Ser 176.

19. A human GH variant, selected from one or more of (locus on hGH in parentheses):
Ile4Val: (N-terminal, within site 2);
Gln22Arg: (helix 1);
Lys41Arg: (loop 1);
Glu56Gly: (in loop region between helices 1 and 2, part of binding site 1);
Arg64Gly: (loop 2);
Lys168Arg: (helix 4);
Lys168Glu; and
Thr175Ala: (helix 4)
as defined with respect to wild type hGH.

20. A human GH variant, which variant comprises the following amino acid substitution with respect to wild type hGH: Glu→Gly 30 [Figure 7, SEQ ID NO:..]

21. A screening method for screening an individual suspected of GH dysfunction, which screening method comprises the steps of:
(a) obtaining a test sample comprising a nucleotide sequence of the human *GH1* gene from the individual; and

(b) comparing a region of the sequence obtained from the test sample with the corresponding region of a predetermined sequence

wherein the predetermined sequence is selected from a variant of *GH1* according to any of claims 12 to 16.

22. A screening method according to claim 21, wherein the test sample comprises genomic DNA.

23. A screening method for screening an individual suspected of GH dysfunction, which screening method comprises the steps of:

(a) obtaining a test sample comprising a nucleotide sequence of the human *GH1* gene or an amino acid sequence encoded thereby from the individual; and

(b) analysing the test sample for the presence of a variant of *GH1* or a GH variant or for the presence of one or more surrogate markers that are indicative of or correlated to the presence of a variant of *GH1* or a GH variant,

wherein the variant of *GH1* or the GH variant exhibits at least one variation when compared to the wild type hGH sequence and is obtainable from a second test sample derived from an individual exhibiting the following criterion:

(i) growth failure defined as a growth pattern [delineated by a series of height measurements; Brook CDG (Ed) Clinical Paediatric Endocrinology 3rd Ed, Chapter 9, p141 (1995, Blackwell Science)] which, when plotted on a standard height chart [Tanner *et al* Arch. Dis. Child 45 755-762 (1970)], predicts an adult height for the individual which is outside the individual's estimated target adult height range, the estimate being based upon the heights of the individual's parents.

24. A screening method according to any one of claims 21 to 23, comprising:

(a) obtaining a first test sample from an individual; and

(b) comparing the *GH1* gene or *GH1* transcript, or fragment therefrom (*eg* cDNA), in the first test sample to the corresponding gene, transcript or fragment of a *GH1* variant obtainable from a second test sample derived from an individual exhibiting the following criterion:

(i) growth failure defined as a growth pattern [delineated by a series of height measurements; Brook CDG (Ed) Clinical Paediatric Endocrinology 3rd Ed, Chapter 9,

p141 (1995, Blackwell Science)] which, when plotted on a standard height chart [Tanner *et al* Arch. Dis. Child 45 755-762 (1970)], predicts an adult height for the individual which is outside the individual's estimated target adult height range, the estimate being based upon the heights of the individual's parents.

25. A screening method according to claim 24, wherein the second test sample is obtainable from an individual exhibiting at least one of the following further criteria:
(ii) height velocity below the 25th centile for age; and/or
(iii) bone age delay according to the Tanner-Whitehouse scale of at least two years when compared with chronological age; and/or
(iv) no other disorder known to cause inclusion in criteria (i) to (iii) above.

26. A screening method according to any of claims 21 to 25 in which simultaneous screens are used either for multiple known mutations or for all possible mutations by hybridization of a labelled sample of DNA (cDNA or genomic DNA derived from the individual) to micro-arrays of mutation-specific oligonucleotide probes immobilised on a solid support.

27. A screening method according to claim 26 in which a chip technology is used, wherein the chip is a miniature parallel analytical device.

28. A kit suitable for use in carrying out a screening method according to any of claims 21 to 27, which kit comprises:
(a) an oligonucleotide having a nucleic acid sequence corresponding to a region of a *GH1* variant, which region incorporates at least one variation from the corresponding wild-type hGH gene sequence; and/or
(b) an oligonucleotide having a nucleic acid sequence corresponding to the wild-type hGH gene sequence in the region specified in (a); and, optionally,
(c) one or more reagents suitable for carrying out PCR for amplifying desired regions of the individual's DNA.

29. A kit according to claim 28, wherein the *GH1* variant comprises at least one of the variants claimed in claims 12 to 16.

30. A kit according to claim 28 or claim 29, wherein kit component (a) comprises a plurality of said oligonucleotides immobilised on a solid support.

31. A kit suitable for use in carrying out a detection method in which the variant is at least one of the variants claimed in claims 12 to 16.

32. A screening method for screening an individual suspected of GH dysfunction, which screening method comprises the steps of:
(a) obtaining a test sample comprising an amino acid sequence encoded by the human *GH1* gene of the individual; and
(b) analysing the test sample for the presence of a GH variant
wherein the GH variant is selected from those according to any one of claims 17 to 20.

33. A screening method according to claim 32, wherein the analysis step (b) is selected from one or more of: conventional protein sequencing methods (such as mass spectroscopy, micro-array analysis, pyrosequencing, *etc*), and/or antibody-based methods of detection (*eg* ELISA).

34. An isolated, purified or recombinant nucleic acid sequence selected from:
(a) a sequence comprising a variant of *GH1* according to any of claims 12 to 16 or encoding a GH variant according to any of claims 17 to 20
(b) a sequence substantially homologous to or that hybridises to sequence (a) under stringent conditions; or
(c) a sequence substantially homologous to or that hybridizes under stringent conditions to the sequence (a) or (b) but for the degeneracy of the genetic code; or
(d) an oligonucleotide specific for any of the sequences (a), (b) or (c).

35. A vector comprising a nucleic acid sequence according to claim 34.

36. A host cell comprising a vector according to claim 35, such as a bacterial host cell.

37. A process for preparing a variant of *GH1* according to any of claims 12 to 16, which process comprises:
(i) culturing a host cell according to claim 36; and
(ii) recovering from the culture medium the variant of *GH1* thereby produced.

38. An amino acid sequence encoded or expressed by a sequence, vector, or cell as defined in any of claims 34 to 37 in culture medium.

39. A composition comprising a variant of *GH1* or a GH variant according to any of claims 12 to 16 or 17 to 20, respectively, in association with a pharmaceutically acceptable carrier therefor.

40. Use of a variant of *GH1* or a GH variant according to any of claims 12 to 16 or 17 to 20, respectively, for a therapeutic, diagnostic or detection method.

41. Use according to claim 40 selected from one of more of: determining binding defects; determining pituitary storage defects; determining susceptibility to a disease, such as diabetes, obesity or infection; treating acromegaly or gigantism conditions associated with lactogenic, diabetogenic, lipolytic and protein anabolic effects; conditions associated with sodium and water retention; metabolic syndromes; mood and sleep disorders; and diagnosing GH dysfunction.

42. Use according to claim 40 of one or more of the variants according to any of claims 12 to 16 in gene therapy.

43. Use according to claim 40 of one or more of the variants according to any of claims 17 to 20 in protein therapy.

44. Use of a variant of GH1 or GH variant according to any of claims 12 to 16 or 17 to 20 respectively, in the preparation of a medicament, diagnostics composition or kit, or detection kit.